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(54) CHONDROITIN SULFATE DERIVED FROM CARTILAGE OF RAJIFORMES AND METHOD FOR PRODUCING THE SAME

(57)Abstract:
PROBLEM TO BE SOLVED: To provide utilization of cartilage of Rajiformes having high-value added and to provide a new biologically active substance.
SOLUTION: A chondroitin sulfate or a mucopolysaccharide protein complex is taken out from the cartilage of Rajiformes. The chondroitin sulfate has a specific structure and a biological activity.

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CLAIMS

[Claim(s)]
[Claim 1] The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Chondroitin sulfate including the disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.
[Claim 2] Disaccharide unit (80.2**16.0) % which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Disaccharide unit (10.0**3.0) % which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Disaccharide unit (2.1**0.7) % which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, Chondroitin sulfate containing disaccharide unit (7.7**2.3) % which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C according to claim 1.
[Claim 3] The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the disaccharide unit containing non-sulfurating N-acetyl-D-galactosamine, the 4th place of C, and the 6th place of C, The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Chondroitin sulfate according to claim 1 or 2 which has the structure which the disaccharide unit including the disaccharide unit which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C connected at random.
[Claim 4] Chondroitin sulfate according to claim 1 to 3 obtained from a ray cartilage.
[Claim 5] (A) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And mucopolysaccharide protein complex containing chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C, and (B) protein.
[Claim 6] (A) Disaccharide unit (80.2**16.0) % which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Disaccharide unit (10.0**3.0) % which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Disaccharide unit (2.1**0.7) % which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, Mucopolysaccharide protein complex containing the chondroitin sulfate containing disaccharide unit (7.7**2.3) % which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C according to claim 1, and (B) protein according to claim 5.
[Claim 7] Mucopolysaccharide protein complex containing the chondroitin sulfate according to claim 5 or 6 obtained from a ray cartilage.
[Claim 8] A ray cartilage is ground, and it processes with an acid, alkali, or an enzyme, and is characterized by carrying out desiccation disintegration of the obtained digestive juices. The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The manufacture approach of chondroitin sulfate including the disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of

C, and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

[Claim 9] A ray cartilage is ground, and it processes with an acid, alkali, or an enzyme, and is characterized by carrying out desiccation disintegration of the obtained digestive juices. (A) The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And the manufacture approach of the mucopolysaccharide protein complex containing chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C, and (B) protein.

[Claim 10] The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C or the drugs containing the mucopolysaccharide protein complex containing said chondroitin sulfate and protein, cosmetics, or food.

[Claim 11] The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C is included as an active principle. The chondroitin sulfate content drugs, cosmetics, or food characterized by having functions, such as reduction control of antitumor action, the object for immunity ******, and a skin collagen.

[Translation done.]

* NOTICES *

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2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DETAILED DESCRIPTION

[Detailed Description of the Invention]
[0001]
[Field of the Invention] This invention relates to the mucopolysaccharide protein complex containing the new chondroitin sulfate obtained considering the cartilage of Rajiformes as a raw material, and this chondroitin sulfate and protein, these manufacture approaches, and the application of those.
[0002]
[Description of the Prior Art] According to the fishing and the aquiculture industry production statistics annual report of the Ministry of Agriculture, Forestry, and Fisheries, the fish catch of Rajiformes belonging to **** fishes exceeds 4000t in the national sum total, and the abbreviation one half is fished in the sea near Hokkaido. However, since it is only the fillet part of a fish with much muscularity which can be used as edible by Rajiformes, it is about 30% of weight at most, and the actual condition is it not being suitable for edible, since other parts' have many cartilages, and not used former sufficiently effectively.
[0003] the shark which the chondroitin sulfate (mucopolysaccharide) which is a physiological active substance is contained in the cartilage of **** fishes so much, and is a kind of **** fishes -- the cartilage of a kind -- or -- and the isolated chondroitin sulfate is used as drugs, a food additive, health food, etc. Moreover, various chondroitin sulfate is extracted and used also from cartilages, such as a whale, a cow, a sheep, a pig, and a bird, besides fishes.
[0004] These chondroitin sulfate consists of disaccharide repeat structure of D-glucuronic acid and the sulfurated N-acetyl galactosamine, within the animal tissue, exists as proteoglycan combined with protein, and is bearing many bioactive operations as an extracellular matrix. Therefore, it is thought that the structure of chondroitin sulfate also changes with animal species or existence parts. actually -- a shark -- the disaccharide unit (chondroitin sulfate C) which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 6th place of C into a cartilage -- The disaccharide unit which becomes a whale cartilage from 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C (chondroitin sulfate A), Specific distribution, such as a disaccharide unit (chondroitin sulfate B) which consists of 1 sulfation N-acetyl-D-galactosamine and D-iduronic acid the 4th place of C, is shown in the pig skin.
[0005] a shark -- there are a lot of cartilages in Rajiformes belonging to the same **** fishes as a kind, and the chondroitin sulfate of a physiological active substance is contained about 5% also in this cartilage. However, the cartilage of Rajiformes was extent used as an organic fertilizer at most with the internal organs of a non-edible portion etc., abolition processing was carried out the actual condition substantially, the presentation structures and those functions of this chondroitin sulfate were not clarified, and industrial up utilization was not carried out.

[0006]
[Problem(s) to be Solved by the Invention] By clarifying the presentation structures and those functions of the new chondroitin sulfate extracted from the cartilage of Rajiformes, and showing the manufacture approach, the technical problem of this invention can be manufactured from trash to a large quantity by low cost, and is to offer the new chondroitin sulfate which can be used for various applications, and its manufacture approach.
[0007]
[Means for Solving the Problem] After this invention person's having ground the ray cartilage and having processed with an acid, alkali, or an enzyme, as a result of inquiring wholeheartedly in view of the above-mentioned technical problem, and carrying out the neutralization demineralization processing of the obtained digestive juices if needed, when this is filtered with the filter press etc. and carries out desiccation solidification

or desiccation disintegration after defecation processing, it came to complete [having the presentation with the unique chondroitin sulfate made into the object, and having a physiological function, and] a header and this invention.

[0008] That is, this invention is to offer the food containing the chondroitin sulfate of the following which has the new structure and the function which are obtained from the Rajiformes cartilage, mucopolysaccharide protein complex, these manufacture approaches, and these etc.

[0009] (1) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Chondroitin sulfate including the disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

[0010] (2) Disaccharide unit (80.2**16.0) % which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Disaccharide unit (10.0**3.0) % which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Disaccharide unit (2.1**0.7) % which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, Chondroitin sulfate given in said 1 containing disaccharide unit (7.7**2.3) % which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

[0011] (3) The disaccharide unit containing non-sulfurating N-acetyl-D-galactosamine, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Chondroitin sulfate given in said 1 or 2 which has the structure which the disaccharide unit including the disaccharide unit which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C connected at random.

(4) Chondroitin sulfate given in either [which is obtained from a ray cartilage / said] 1 thru/or 3.

[0012] (5) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of (A) C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And mucopolysaccharide protein complex containing chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C, and (B) protein.

[0013] (6) Disaccharide unit (80.2**16.0) % which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of (A) C, Disaccharide unit (10.0**3.0) % which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Disaccharide unit (2.1**0.7) % which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, Mucopolysaccharide protein complex given in said 5 which contains the chondroitin sulfate of a publication, and (B) protein in said 1 containing disaccharide unit (7.7**2.3) % which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

(7) Mucopolysaccharide protein complex which contains the chondroitin sulfate of a publication in said 5 or 6 obtained from a ray cartilage.

[0014] (8) A ray cartilage is ground, and it processes with an acid, alkali, or an enzyme, and is characterized by carrying out desiccation disintegration of the obtained digestive juices. The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The manufacture approach of chondroitin sulfate including the disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

[0015] (9) A ray cartilage is ground, and it processes with an acid, alkali, or an enzyme, and is characterized by carrying out desiccation disintegration of the obtained digestive juices. (A) The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-

galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And the manufacture approach of the mucopolysaccharide protein complex containing chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C, and (B) protein.

[0016] (10) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C or the drugs containing the mucopolysaccharide protein complex containing said chondroitin sulfate and protein, cosmetics, or food.

[0017] (11) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C is included as an active principle. The chondroitin sulfate content drugs, cosmetics, or food characterized by having functions, such as reduction control of antitumor action, the object for immunity ******, and a skin collagen.

[0018]

[Embodiment of the Invention] The chondroitin sulfate of this invention includes the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C are included. Moreover, the mucopolysaccharide protein complex of this invention is complex of the above-mentioned chondroitin sulfate and protein.

[0019] the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C which is the principal component of the chondroitin sulfate of this invention -- a shark -- what was refined from the cartilage became whether to be Toshiaki Kon et al. The chondroitin in a cartilage is chondroitin (chondroitin sulfate C) which mainly consists of disaccharide units which consist of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 6th place of C. a shark -- The disaccharide unit which becomes a whale cartilage from 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, Although it is known that the chondroitin (respectively chondroitin sulfate A, chondroitin sulfate B) which includes many disaccharide units which consist of 1 sulfation N-acetyl-D-galactosamine and D-iduronic acid the 4th place of C in a pig skin exists mostly, respectively The thing of the rate of the after-mentioned [the abundance ratio of the disaccharide which constitutes chondroitin sulfate] is not known conventionally specifically, including mostly the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C.

[0020] The chondroitin sulfate and the mucopolysaccharide protein complex of this invention can be preferably obtained from the cartilage of Rajiformes. Although especially the class of Rajiformes is not limited, HIRATAEI, a whip ray, ITOMAKIEI, TSUNOKASUBE, SOKOGANGIEI, a skate, glasses Raja, DOBUKASUBE, common Raja, etc. can be used, for example. As for the approach of carrying out separation recovery of the cartilage of Rajiformes used as a raw material, the approach of a publication etc. is mentioned to an application for patent 2001-124634.

[0021] The cartilage separated from the fish is broken finely and it processes with an acid, alkali, or an enzyme. Grinding of a cartilage should just be extent which an extracting solvent permeates effectively. For example, it is desirable to grind or less [1cm] to three using a meat chopper etc. The range of acid treatment of pH 1-5 is desirable. The range of alkali treatment of pH 10-14 is desirable. An enzyme can use dialytic ferments, such as a protease.

[0022] After carrying out neutralization demineralization processing, filtering if needed and removing solid content, desiccation solidification or desiccation disintegration of the obtained processing object is carried out. Filtration is taken as a clear extract using a filter press, a screen mesh, a cartridge filter, etc. In filtration, the ion

in the extract which cannot be removed may be removed with the demineralization processing performed to an extract by impressing an electrical potential difference, an ion exchange column, etc. The chondroitin sulfate or the mucopolysaccharide protein complex made into the object by carrying out desiccation solidification or desiccation disintegration after filtration is obtained. What is necessary is for enzyme digestion to decompose protein, to perform precipitate by ethanol, ion exchange column processing, filtration by the membrane filter, etc., and just to remove amino acid and unnecessary aminosugar thoroughly, in order to obtain especially the chondroitin sulfate of a high grade.

[0023] Thus, typically, the prepared chondroitin sulfate has the following presentations.

(a) disaccharide unit: which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C -- disaccharide unit: which consists of (b) non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid 80.2**16.0% -- 10.0**3.0% (c) -- disaccharide unit: which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C -- 2.1**0.7% and (d) -- disaccharide unit:7.7**2.3% which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

% is mol% of each configuration unit here.

[0024] The mucopolysaccharide protein complex with which this invention persons contain this new chondroitin sulfate or this further found out having anticancerous to mouse colon cancer (Colon 26) and mouse mammary gland cancer (calcium 755), and having the anti-MRSA operation by immunity activation, and the skin collagen lowering depressant action by the vitamin C deficiency guinea pig. Therefore, the mucopolysaccharide protein complex containing the chondroitin sulfate of this invention, and this For example, it has a function as an immunity force enhancement agent to elderly people, the invalid, those whose physical strength declined. Like conventional chondroitin sulfate, moreover, an anti-inflammatory agent, a moisturizer, joint lubricant, It can utilize for very various applications, such as asthenopia palliative and a metabolic turnover improvement agent of the skin, and the new function by the unique structure can be further applied to large range, such as drugs, cosmetics, food, and macromolecule industry.

[0025]

[Example] Hereafter, although an example and the example of a trial are given and explained, this invention is not limited to the following publications.

[0026] Example 1: Enzyme processing was performed by putting 106kg of ray cartilage grinding objects, and protease 210g into the manufacture clinodiagonal kneader (200L capacity) of ray cartilage origin chondroitin sulfate (mucopolysaccharide protein complex), and stirring at 59 degrees C for 2 hours. It heated at 90 degrees C succeedingly, it stirred for 15 minutes, deactivation of the enzyme was carried out, 5kg of filter aids was added to this, and the filter press performed pressure filtration after homogenization. In addition, the used pressure filtration equipment is a Makino M8-S form (filtration area: 0.27m2 and filtration volume:2.7L, construction material:SUS304). Next, the obtained extract was heated at 90 degrees C, for 10 minutes, after heat sterilization, spray drying was carried out with the disk mold spray dryer (Made in Sakamoto Research Institute DA220-10S), and 10.6kg of white powder of chondroitin sulfate (mucopolysaccharide protein complex is included.) was obtained. Spray drying conditions were performed by a atomization method:phi90mm revolution pin mold disk, 12,000rpm, inlet-port hot-blast-temperature:185 degree C, outlet exhaust-air-temperature:90 degree C, and drying-room internal pressure:-10mmH2O.

[0027] Example 2: Purification of ray cartilage origin chondroitin sulfate and its structural-analysis purification chondroitin sulfate were obtained with the following procedures, and structural analysis was presented with them.

1. Cleaning 1 The 50g ray cartilage drying grinding article was put into the 1000ml beaker, acetone 500ml was added, and it agitated for 10 minutes.

2) It was left for 5 minutes and the supernatant was thrown away.

3) Actuation of 1-2 was repeated further 3 times.

4) Remaining sediment was dried with the vacuum desiccator.

[0028] 2. Alkali treatment 1 44.5g of degreased ray cartilage powder was dissolved in 0.2 M NaOH 800ml.

2) It agitated in the water bath of 37 degree C for 3 hours.

3) The acetic acid neutralized to pH7.

3. Proteolysis oxygenation 1 100ml (pH7.8) of 0.2 M Tris-HCl buffer solutions was added.

2) Calcium acetate was added so that it might be set to final concentration 0.02M.

3) 50ml of methanols was added for preservation from decay.

4) 500mg (AKUCHINAZE E Kaken Pharmaceutical) of proteolysis oxygen was added.
5) It agitated slowly in the water bath of 37 degree C for 48 hours.
[0029] 4. Ethanol precipitate 1 Centrifugal separation of the digestive juices was carried out at 4 degrees C for [10,000rpmx] 30 minutes.
2) Attraction **** of the supernatant was carried out using 0.45-micrometer membrane filter.
3) Final concentration is 0.05M to a supernatant. Calcium acetate was added so that it might become.
4) It is pH4.5 with an acetic acid. It adjusted.
5) Twice as many ethanol as this was dropped and it was left also as that [4-degree C] for 48 hours.
[0030] 5. Washing and desiccation 1 of precipitate Centrifugal separation of the ethanol liquid was carried out at 4 degrees C for [7,000rpmx] 30 minutes.
2) Precipitate was collected, ethanol 3000ml was added 80%, and it agitated slowly at 4 degrees C for 12 hours.
3) Centrifugal separation was carried out at 4 degrees C for [1,000rpmx] 30 minutes.
4) Actuation of 2-3 was repeated.
5) Ethanol 2000ml was added to precipitate 100%, and it agitated slowly at 4 degrees C for 6 hours.
6) Centrifugal separation was carried out at 4 degrees C for [1,000rpmx] 30 minutes.
7) Precipitate was dried in reduced pressure DEJIGETA and rough chondroitin sulfate was obtained.
[0031] 6. Adjustment 1 of DOWEX50Wx2 cation-exchange column 200ml of DOWEX50Wx2 cation exchange resin is agitated in 0.5 N HCl for 2 hours, and they are after washing and 0.5 M NaOH with milli queue water. It agitated in inside for 2 hours.
2) 1 was repeated 3 times, it considered as the form of proton maintenance of exchange resin, and milli queue water washed.
3) The column was put into the 500ml beaker and it considered as the batch type reaction tank.
[0032] 7. DOWEX50Wx2 cation-exchange-resin processing 1 The obtained rough chondroitin sulfate was dissolved in very little milli queue water.
2) The sample produced by 1 was added to the batch type reaction tank, and it agitated to it for 1.5 hours. The solutions after a reaction were collected, and the penetrant remover also set and collected resin after washing with 800ml milli queue water further.
4) Recovery liquid was immediately neutralized to pH7 in 1NNaOH.
8. Purification 1 Dialysis (molecular-weight cut-off: 10kD) of the neutralization liquid was carried out for three days in deionized water.
2) It condensed in optimum dose in the evaporator.
3) It freeze-dried after **** with the 0.2micrometer membrane filter, and considered as purification chondroitin sulfate.
[0033] Structural analysis was presented with the purification chondroitin sulfate obtained as mentioned above. In addition, the mucopolysaccharide protein complex (Lot.1) prepared in the example 1 other than purification chondroitin sulfate (Lot.2 of a table 1) and three kinds of chondroitin sulfate (Lot.3-5) from which the origin differs were prepared as shown in a table 1 for the comparison. Lot.3 used the salmon cartilagines nasi as the raw material, and also they are prepared like Lot.2. It reaches Lot.4 and 5 is the commercial Seikagaku preparation, Lot.4 are chondroitin sulfate A and Lot.5 are chondroitin sulfate C.
[0034]
[A table 1]

表１：構造解析に用いた試料

Lot.1	エイ軟骨由来コンドロイチン硫酸を含むムコ多糖タンパク複合体
Lot.2	エイ軟骨由来コンドロイチン硫酸
Lot.3	サケ鼻軟骨由来コンドロイチン硫酸
Lot.4	クジラ軟骨由来コンドロイチン硫酸
Lot.5	サメ軟骨由来コンドロイチン硫酸

[0035] Specified quantity weighing capacity of amino-acid-analysis each sample (Lot.1-2) was carried out, respectively, and it hydrolyzed according to about 100 degrees C and the conditions of 6 hours using 2.4 N-HCl. The quantum of this was carried out with amino-acid-analysis equipment (JLC-500V, JEOL make). The analysis result was shown in drawing 1 . Gly (glycine), HyPro (hydroxyproline), and Pro (proline) which are looked at by Lot.1 show existence of a collagen. Moreover, in Lot.2 to which purification processing was performed, since any amino acid other than GalNH2 was not seen, the aminosugar which constitutes Rajiformes cartilage origin

chondroitin sulfate has ******(ed) that other amino acid and aminosugar were thoroughly removable with purification processing of that it is only a galactosamine kind and this experiment.

[0036] The 400MHz 1 H-NMR spectrum of 1 H-NMR measurement each sample (Lot.1-5) was measured. The measurement result was shown in drawing 2 . The signal of the protein origin has been checked near 0.8-2.5 ppm at Lot 1. It is thought that this protein is mainly a collagen. The spectrum of the Rajiformes cartilage origin chondroitin sulfate of Lot 2 has checked that it was close to the spectrum of the salmon cartilagines-nasi origin chondroitin sulfate of Lot 3 rather than the spectrum of Lot4 and Lot5. When this Lot 2 was looked at in the detail, the signal (GalNAC-4S) of the proton of the 4th place of the N-acetyl galactosamine considered that the sulfuric-acid radical has joined together has checked near 4.8 ppm. Moreover, although integrated intensity fell, the signal (GalNAC-6S) of the proton of the 6th place has checked it near 4.1 ppm similarly.

[0037] Disaccharide HPLC analysis 1 Chondroitin sulfate dialytic ferment (Chodroitinase ACII) 5unit was dissolved in 500micro (pH6.0) of 0.02M sodium acetate buffer solutions I.

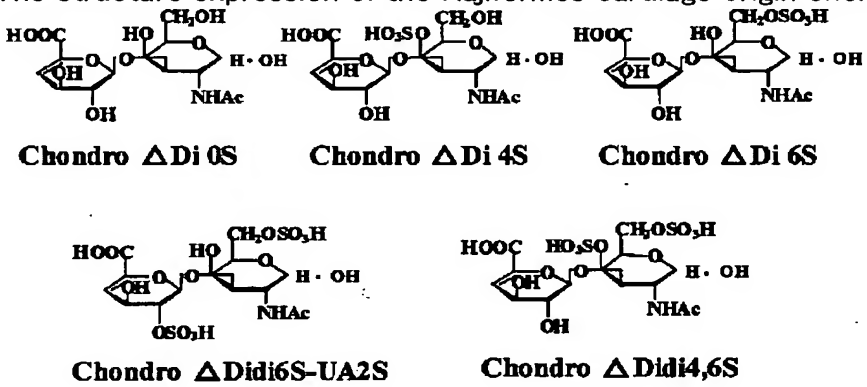
2) The chondroitin sulfate dialytic ferment (Chodroitinase ACII) solution which dissolved in 500micro (pH6.0) of 0.02M sodium acetate buffer solutions I, and adjusted Rajiformes cartilage origin chondroitin sulfate (sample Lot.2) by 1 was added, and it stirred at 37 degrees C for 24 hours.

3) At-long-intervals alignment separation of this was carried out for 10,000rpmx 10 minutes, and supernatants were collected and it freeze-dried.

4) HPLC analysis of the obtained disaccharide oligomer was performed and the presentation of a configuration disaccharide was computed from the obtained chromatogram. The presentation ratio of a configuration partial saturation disaccharide was shown in a table 2. In addition, each structure expression is as follows. In addition, the conditions of HPLC analysis were shown in a table 3.

[0038]

[Formula 1] The structure expression of the Rajiformes cartilage origin chondroitin sulfate configuration disaccharide



[0039]

[A table 2]

表２ 不飽和二糖構成比（%）

Condro ΔDi 0S	10.0
Condro ΔDi 4S + Condro ΔDi 6S	2.1
Condro Δdidi 6S-UA2S	80.2
Condro Δdidi 4,6S	7.7

[0040]

[A table 3]

表3 HPLC分析条件

カラム	GL Science NUCLEOSIL 100-5NH_2(4.6×250mm)
溶出液	2%アセトニトリル, 12-408mM NaH_2PO_4 gradient/MiliQ water
流速	0.6ml/分
試料容積	40μl
温度	40℃
検出方法	UV 232nm
測定システム	HPLC(Shimadzu Corporation)

[0041] The structure of Rajiformes cartilage origin chondroitin sulfate became clear [that 2 sugar repeat unit which consists of sulfation glucuronic acid the 2nd place has the 6th place of the very characteristic presentation which occupies the 80% or more with sulfation N-acetyl galactosamine]. the above-mentioned passage -- a shark -- it is supposed that it is mainly cartilage origin chondroitin sulfate chondroitin sulfate C, and the chondroitin sulfate of this invention has the 6th place of sulfation N-acetyl galactosamine and the characteristic structure which includes specifically many 2 sugar repeat units which consist of sulfation glucuronic acid the 2nd place.

[0042] Example 3: The Rajiformes cartilage origin chondroitin sulfate or the mucopolysaccharide protein complex (following, KCS) of functional this invention of ray cartilage origin chondroitin sulfate has unique structure as compared with the structure of the conventional known. Therefore, possibility of having the new bioactive operation which is not in other chondroitin sulfate can be considered. In order to examine this, the animal trial performed functional assessment by making into the charge of a sample offering the mucopolysaccharide protein complex containing the ray cartilage origin chondroitin sulfate prepared in the example 1.

[0043] Mixed feed administration of the KCS added to with an anticancerous of 4 weeks old BDF1 and CDF1 mouse at feed was carried out by continuation for 42 days. The tumor cell was transplanted at a rate of 1x106 cells / 0.05ml / mouse on the 28th (calcium 755 are BDF1 and Colon26 are CDF1), and administration of KCS was continued after transplantation. Cervical-vertebra dislocation of the mouse was carried out under anesthesia, a neoplasm was extracted, and wet weight was measured on the next day [KCS administration termination] (the 43rd day). The experimental plot was shown in a table 4. The multiplex comparison of Dunnett performed significant difference assay. The obtained result was shown in drawing 3 and drawing 4 . About mouse mammary gland cancer (calcium 755), significant antitumor action was statistically accepted [colon cancer / (Colon 26) / more than KCS 6% and / mouse] by administration beyond KCS 3% as compared with the control group.

[0044]

[A table 4]

表4 抗腫瘍試験の群構成

群	添加量	投与日数	動物数（匹）	
	%（混餌）	日	Ca755	Colon26
対照	0	42	8	8
KCS	1	42	8	8
〃	3	42	8	8
〃	6	42	8	8
〃	9	42	8	8

[0045] Mixed feed administration of the KCS added at feed to the ddY system male mouse of 4 weeks old of anti-MRSA (methicillin resistant Staphylococcus aureus) was carried out by continuation for 42 days. To the mouse, at a rate of 6mg / animal, it continued for 1 three days once per day, and cyclophosphamide (EX) was injected intraperitoneally from the 25th day. You made it infected by administering MRSA intravenously at a rate of 107 piece / animal at the next day which prescribed the cyclophosphamide on the 3rd for the patient. It observed until the animal died from the MRSA inoculation day, and survival time was computed. KCS continued administration within the limit of ten days until the animal died. The experimental plot was shown in a table 5. Significant difference assay performed survival analysis assay of Kaplan-Meier. The obtained result was shown in

drawing 5 . In the result of survival analysis assay, the significant difference was not accepted in each test section. However, although it was 50% in the control group when seen with the survival rate of three days after, with the mouse made to take in KCS6%, 87.5%, with the mouse made to take in KCS 9%, it is 100% and some prolongation-of-life effectiveness was seen.

[0046]

[A table 5]

表5 抗MRSA試験の群構成

群	添加量 % (混餌)	投与日数 日	動物数 匹
対照	0	死亡するまで (最大10日)	8
KCS	1	死亡するまで (最大10日)	8
〃	3	死亡するまで (最大10日)	8
〃	6	死亡するまで (最大10日)	8
〃	9	死亡するまで (最大10日)	8

[0047] The group non-taken a measure gave the guinea pig of 4 weeks old of moistness of the skin, and normal feed and a control group gave vitamin C deficiency feed. The feed which blended KCS with vitamin C deficiency feed was given to the KCS group, and it bred for 28 days, respectively. The regions-of-back skin was exfoliated after breeding for 28 days, and the collagen content was measured. Collagen content measurement ground the skin dried at 60 degrees C overnight, and hydrolyzed it 110 degrees C for 24 hours using the heating block (Yamato Science, HF21) in 6M hydrochloric acid. The hydroxyproline (Hyp) content was measured with the chloramine-T method by making this into a sample. The experimental plot was shown in a table 6. The multiplex comparison of Dunnett performed significant difference assay. The obtained result was shown in drawing 6 . Collagens are the main constituents in the skin and are contained 70 to 80% per unit dry weight in dermis. Moreover, since reduction of a skin collagen content is also known with aging, a collagen maintains the elasticity of the skin and it is thought that it has functions, such as preventing a wrinkling. As a result of breeding a guinea pig with vitamin C deficiency feed, the collagen content in the skin decreased about 50%. On the other hand, the control inclination of collagen content lowering was accepted with the guinea pig made to take in KCS 3%.

[0048]

[A table 6]

表6 皮膚機能に関する試験の群構成

群	添加量 % (混餌)	投与日数 日	動物数 匹
無処置	0	28	5
対照	0	28	5
KCS	1	28	5
	3	28	5

[0049]

[Effect of the Invention] The mucopolysaccharide protein complex containing the chondroitin sulfate of this invention, and this For example, it has a function as an immunity force enhancement agent to elderly people, the invalid, those whose physical strength declined. Like conventional chondroitin sulfate, moreover, an anti-inflammatory agent, a moisturizer, joint lubricant, It can utilize for very various applications, such as asthenopia palliative and a metabolic turnover improvement agent of the skin, and the new function by the unique structure may be able to be further applied to large range, such as drugs, cosmetics, food, and macromolecule industry.

[Translation done.]

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TECHNICAL FIELD

[Field of the Invention] This invention relates to the mucopolysaccharide protein complex containing the new chondroitin sulfate obtained considering the cartilage of Rajiformes as a raw material, and this chondroitin sulfate and protein, these manufacture approaches, and the application of those.

[Translation done.]

PRIOR ART

[Description of the Prior Art] According to the fishing and the aquiculture industry production statistics annual report of the Ministry of Agriculture, Forestry, and Fisheries, the fish catch of Rajiformes belonging to **** fishes exceeds 4000t in the national sum total, and the abbreviation one half is fished in the sea near Hokkaido. However, since it is only the fillet part of a fish with much muscularity which can be used as edible by Rajiformes, it is about 30% of weight at most, and the actual condition is it not being suitable for edible, since other parts' have many cartilages, and not used former sufficiently effectively.
[0003] the shark which the chondroitin sulfate (mucopolysaccharide) which is a physiological active substance is contained in the cartilage of **** fishes so much, and is a kind of **** fishes -- the cartilage of a kind -- or -- and the isolated chondroitin sulfate is used as drugs, a food additive, health food, etc. Moreover, various chondroitin sulfate is extracted and used also from cartilages, such as a whale, a cow, a sheep, a pig, and a bird, besides fishes.
[0004] These chondroitin sulfate consists of disaccharide repeat structure of D-glucuronic acid and the sulfurated N-acetyl galactosamine, within the animal tissue, exists as proteoglycan combined with protein, and is bearing many bioactive operations as an extracellular matrix. Therefore, it is thought that the structure of chondroitin sulfate also changes with animal species or existence parts. actually -- a shark -- the disaccharide unit (chondroitin sulfate C) which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 6th place of C into a cartilage -- The disaccharide unit which becomes a whale cartilage from 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C (chondroitin sulfate A), Specific distribution, such as a disaccharide unit (chondroitin sulfate B) which consists of 1 sulfation N-acetyl-D-galactosamine and D-iduronic acid the 4th place of C, is shown in the pig skin.
[0005] a shark -- there are a lot of cartilages in Rajiformes belonging to the same **** fishes as a kind, and the chondroitin sulfate of a physiological active substance is contained about 5% also in this cartilage. However, the cartilage of Rajiformes was extent used as an organic fertilizer at most with the internal organs of a non-edible portion etc., abolition processing was carried out the actual condition substantially, the presentation structures and those functions of this chondroitin sulfate were not clarified, and industrial up utilization was not carried out.

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EFFECT OF THE INVENTION

[Effect of the Invention] The mucopolysaccharide protein complex containing the chondroitin sulfate of this invention, and this For example, it has a function as an immunity force enhancement agent to elderly people, the invalid, those whose physical strength declined. Like conventional chondroitin sulfate, moreover, an anti-inflammatory agent, a moisturizer, joint lubricant, It can utilize for very various applications, such as asthenopia palliative and a metabolic turnover improvement agent of the skin, and the new function by the unique structure may be able to be further applied to large range, such as drugs, cosmetics, food, and macromolecule industry.

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TECHNICAL PROBLEM

[Problem(s) to be Solved by the Invention] By clarifying the presentation structures and those functions of the new chondroitin sulfate extracted from the cartilage of Rajiformes, and showing the manufacture approach, the technical problem of this invention can be manufactured from trash to a large quantity by low cost, and is to offer the new chondroitin sulfate which can be used for various applications, and its manufacture approach.

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MEANS

[Means for Solving the Problem] After this invention person's having ground the ray cartilage and having processed with an acid, alkali, or an enzyme, as a result of inquiring wholeheartedly in view of the above-mentioned technical problem, and carrying out the neutralization demineralization processing of the obtained digestive juices if needed, when this is filtered with the filter press etc. and carries out desiccation solidification or desiccation disintegration after defecation processing, it came to complete [having the presentation with the unique chondroitin sulfate made into the object, and having a physiological function, and] a header and this invention.
[0008] That is, this invention is to offer the food containing the chondroitin sulfate of the following which has the new structure and the function which are obtained from the Rajiformes cartilage, mucopolysaccharide protein complex, these manufacture approaches, and these etc.
[0009] (1) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Chondroitin sulfate including the disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.
[0010] (2) Disaccharide unit (80.2**16.0) % which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Disaccharide unit (10.0**3.0) % which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Disaccharide unit (2.1**0.7) % which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, Chondroitin sulfate given in said 1 containing disaccharide unit (7.7**2.3) % which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.
[0011] (3) The disaccharide unit containing non-sulfurating N-acetyl-D-galactosamine, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C, Chondroitin sulfate given in said 1 or 2 which has the structure which the disaccharide unit including the disaccharide unit which contains disulfuric acid-ized N-acetyl-D-galactosamine C4 and the 6th place of C connected at random.
(4) Chondroitin sulfate given in either [which is obtained from a ray cartilage / said] 1 thru/or 3.
[0012] (5) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of (A) C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And mucopolysaccharide protein complex containing chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C, and (B) protein.
[0013] (6) Disaccharide unit (80.2**16.0) % which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of (A) C, Disaccharide unit (10.0**3.0) % which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, Disaccharide unit (2.1**0.7) % which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, Mucopolysaccharide protein complex given in said 5 which contains the chondroitin sulfate of a publication, and (B) protein in said 1 containing disaccharide unit (7.7**2.3) % which consists of disulfuric acid-

ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.
(7) Mucopolysaccharide protein complex which contains the chondroitin sulfate of a publication in said 5 or 6 obtained from a ray cartilage.
[0014] (8) A ray cartilage is ground, and it processes with an acid, alkali, or an enzyme, and is characterized by carrying out desiccation disintegration of the obtained digestive juices. The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The manufacture approach of chondroitin sulfate including the disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.
[0015] (9) A ray cartilage is ground, and it processes with an acid, alkali, or an enzyme, and is characterized by carrying out desiccation disintegration of the obtained digestive juices. (A) The disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C is included 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And the manufacture approach of the mucopolysaccharide protein complex containing chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C, and (B) protein.
[0016] (10) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C or the drugs containing the mucopolysaccharide protein complex containing said chondroitin sulfate and protein, cosmetics, or food.
[0017] (11) Include the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C, And chondroitin sulfate including the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C is included as an active principle. The chondroitin sulfate content drugs, cosmetics, or food characterized by having functions, such as reduction control of antitumor action, the object for immunity ******, and a skin collagen.
[0018]
[Embodiment of the Invention] The chondroitin sulfate of this invention includes the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C 60% or more. Furthermore, the disaccharide unit which consists of non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid, The disaccharide unit which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C and the 6th place of C, and the disaccharide unit which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C are included. Moreover, the mucopolysaccharide protein complex of this invention is complex of the above-mentioned chondroitin sulfate and protein.
[0019] the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C which is the principal component of the chondroitin sulfate of this invention -- a shark -- what was refined from the cartilage became whether to be Toshiaki Kon et al. The chondroitin in a cartilage is chondroitin (chondroitin sulfate C) which mainly consists of disaccharide units which consist of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 6th place of C. a shark -- The disaccharide unit which becomes a whale cartilage from 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, Although it is known that the chondroitin (respectively chondroitin sulfate A, chondroitin sulfate B) which includes many disaccharide units which consist of 1 sulfation N-acetyl-D-galactosamine and D-iduronic acid the 4th place of C in a pig skin exists mostly, respectively The thing of the rate of the after-mentioned [the abundance ratio of the disaccharide which constitutes chondroitin sulfate] is not known

conventionally specifically, including mostly the disaccharide unit which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C.

[0020] The chondroitin sulfate and the mucopolysaccharide protein complex of this invention can be preferably obtained from the cartilage of Rajiformes. Although especially the class of Rajiformes is not limited, HIRATAEI, a whip ray, ITOMAKIEI, TSUNOKASUBE, SOKOGANGIEI, a skate, glasses Raja, DOBUKASUBE, common Raja, etc. can be used, for example. As for the approach of carrying out separation recovery of the cartilage of Rajiformes used as a raw material, the approach of a publication etc. is mentioned to an application for patent 2001-124634.

[0021] The cartilage separated from the fish is broken finely and it processes with an acid, alkali, or an enzyme. Grinding of a cartilage should just be extent which an extracting solvent permeates effectively. For example, it is desirable to grind or less [1cm] to three using a meat chopper etc. The range of acid treatment of pH 1-5 is desirable. The range of alkali treatment of pH 10-14 is desirable. An enzyme can use dialytic ferments, such as a protease.

[0022] After carrying out neutralization demineralization processing, filtering if needed and removing solid content, desiccation solidification or desiccation disintegration of the obtained processing object is carried out. Filtration is taken as a clear extract using a filter press, a screen mesh, a cartridge filter, etc. In filtration, the ion in the extract which cannot be removed may be removed with the demineralization processing performed to an extract by impressing an electrical potential difference, an ion exchange column, etc. The chondroitin sulfate or the mucopolysaccharide protein complex made into the object by carrying out desiccation solidification or desiccation disintegration after filtration is obtained. What is necessary is for enzyme digestion to decompose protein, to perform precipitate by ethanol, ion exchange column processing, filtration by the membrane filter, etc., and just to remove amino acid and unnecessary aminosugar thoroughly, in order to obtain especially the chondroitin sulfate of a high grade.

[0023] Thus, typically, the prepared chondroitin sulfate has the following presentations.

(a) disaccharide unit: which consists of 1 sulfation D-glucuronic acid the 6th place 1 sulfation N-acetyl-D-galactosamine and the 2nd place of C of C -- disaccharide unit: which consists of (b) non-sulfurating N-acetyl-D-galactosamine and D-glucuronic acid 80.2**16.0% -- 10.0**3.0% (c) -- disaccharide unit: which consists of 1 sulfation N-acetyl-D-galactosamine and D-glucuronic acid the 4th place of C, and the 6th place of C -- 2.1**0.7% and (d) -- disaccharide unit:7.7**2.3% which consists of disulfuric acid-ized N-acetyl-D-galactosamine and D-glucuronic acid C4 and the 6th place of C.

% is mol% of each configuration unit here.

[0024] The mucopolysaccharide protein complex with which this invention persons contain this new chondroitin sulfate or this further found out having anticancerous to mouse colon cancer (Colon 26) and mouse mammary gland cancer (calcium 755), and having the anti-MRSA operation by immunity activation, and the skin collagen lowering depressant action by the vitamin C deficiency guinea pig. Therefore, the mucopolysaccharide protein complex containing the chondroitin sulfate of this invention, and this For example, it has a function as an immunity force enhancement agent to elderly people, the invalid, those whose physical strength declined. Like conventional chondroitin sulfate, moreover, an anti-inflammatory agent, a moisturizer, joint lubricant, It can utilize for very various applications, such as asthenopia palliative and a metabolic turnover improvement agent of the skin, and the new function by the unique structure can be further applied to large range, such as drugs, cosmetics, food, and macromolecule industry.

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EXAMPLE

[Example] Hereafter, although an example and the example of a trial are given and explained, this invention is not limited to the following publications.
[0026] Example 1: Enzyme processing was performed by putting 106kg of ray cartilage grinding objects, and protease 210g into the manufacture clinodiagonal kneader (200L capacity) of ray cartilage origin chondroitin sulfate (mucopolysaccharide protein complex), and stirring at 59 degrees C for 2 hours. It heated at 90 degrees C succeedingly, it stirred for 15 minutes, deactivation of the enzyme was carried out, 5kg of filter aids was added to this, and the filter press performed pressure filtration after homogenization. In addition, the used pressure filtration equipment is a Makino M8-S form (filtration area: 0.27m2 and filtration volume:2.7L, construction material:SUS304). Next, the obtained extract was heated at 90 degrees C, for 10 minutes, after heat sterilization, spray drying was carried out with the disk mold spray dryer (Made in Sakamoto Research Institute DA220-10S), and 10.6kg of white powder of chondroitin sulfate (mucopolysaccharide protein complex is included.) was obtained. Spray drying conditions were performed by a atomization method:phi90mm revolution pin mold disk, 12,000rpm, inlet-port hot-blast-temperature:185 degree C, outlet exhaust-air-temperature:90 degree C, and drying-room internal pressure:-10mmH2O.
[0027] Example 2: Purification of ray cartilage origin chondroitin sulfate and its structural-analysis purification chondroitin sulfate were obtained with the following procedures, and structural analysis was presented with them.
1. Cleaning 1 The 50g ray cartilage drying grinding article was put into the 1000ml beaker, acetone 500ml was added, and it agitated for 10 minutes.
2) It was left for 5 minutes and the supernatant was thrown away.
3) Actuation of 1-2 was repeated further 3 times.
4) Remaining sediment was dried with the vacuum desiccator.
[0028] 2. Alkali treatment 1 44.5g of degreased ray cartilage powder was dissolved in 0.2 M NaOH 800ml.
2) It agitated in the water bath of 37 degree C for 3 hours.
3) The acetic acid neutralized to pH7.
3. Proteolysis oxygenation 1 100ml (pH7.8) of 0.2 M Tris-HCl buffer solutions was added.
2) Calcium acetate was added so that it might be set to final concentration 0.02M.
3) 50ml of methanols was added for preservation from decay.
4) 500mg (AKUCHINAZE E Kaken Pharmaceutical) of proteolysis oxygen was added.
5) It agitated slowly in the water bath of 37 degree C for 48 hours.
[0029] 4. Ethanol precipitate 1 Centrifugal separation of the digestive juices was carried out at 4 degrees C for [10,000rpmx] 30 minutes.
2) Attraction **** of the supernatant was carried out using 0.45-micrometer membrane filter.
3) Final concentration is 0.05M to a supernatant. Calcium acetate was added so that it might become.
4) It is pH4.5 with an acetic acid. It adjusted.
5) Twice as many ethanol as this was dropped and it was left also as that [4-degree C] for 48 hours.
[0030] 5. Washing and desiccation 1 of precipitate Centrifugal separation of the ethanol liquid was carried out at 4 degrees C for [7,000rpmx] 30 minutes.
2) Precipitate was collected, ethanol 3000ml was added 80%, and it agitated slowly at 4 degrees C for 12 hours.
3) Centrifugal separation was carried out at 4 degrees C for [1,000rpmx] 30 minutes.
4) Actuation of 2-3 was repeated.
5) Ethanol 2000ml was added to precipitate 100%, and it agitated slowly at 4 degrees C for 6 hours.

6) Centrifugal separation was carried out at 4 degrees C for [1,000rpmx] 30 minutes.
7) Precipitate was dried in reduced pressure DEJIGETA and rough chondroitin sulfate was obtained.
[0031] 6. Adjustment 1 of DOWEX50Wx2 cation-exchange column 200ml of DOWEX50Wx2 cation exchange resin is agitated in 0.5 N HCl for 2 hours, and they are after washing and 0.5 M NaOH with milli queue water. It agitated in inside for 2 hours.
2) 1 was repeated 3 times, it considered as the form of proton maintenance of exchange resin, and milli queue water washed.
3) The column was put into the 500ml beaker and it considered as the batch type reaction tank.
[0032] 7. DOWEX50Wx2 cation-exchange-resin processing 1 The obtained rough chondroitin sulfate was dissolved in very little milli queue water.
2) The sample produced by 1 was added to the batch type reaction tank, and it agitated to it for 1.5 hours. The solutions after a reaction were collected, and the penetrant remover also set and collected resin after washing with 800ml milli queue water further.
4) Recovery liquid was immediately neutralized to pH7 in 1NNaOH.
8. Purification 1 Dialysis (molecular-weight cut-off: 10kD) of the neutralization liquid was carried out for three days in deionized water.
2) It condensed in optimum dose in the evaporator.
3) It freeze-dried after **** with the 0.2micrometer membrane filter, and considered as purification chondroitin sulfate.
[0033] Structural analysis was presented with the purification chondroitin sulfate obtained as mentioned above. In addition, the mucopolysaccharide protein complex (Lot.1) prepared in the example 1 other than purification chondroitin sulfate (Lot.2 of a table 1) and three kinds of chondroitin sulfate (Lot.3-5) from which the origin differs were prepared as shown in a table 1 for the comparison. Lot.3 used the salmon cartilagines nasi as the raw material, and also they are prepared like Lot.2. It reaches Lot.4 and 5 is the commercial Seikagaku preparation, Lot.4 are chondroitin sulfate A and Lot.5 are chondroitin sulfate C.
[0034]
[A table 1]

表１：構造解析に用いた試料

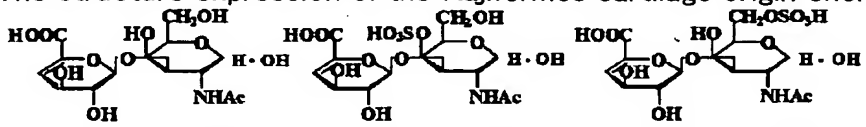
Lot.1	エイ軟骨由来コンドロイチン硫酸を含むムコ多糖タンパク複合体
Lot.2	エイ軟骨由来コンドロイチン硫酸
Lot.3	サケ鼻軟骨由来コンドロイチン硫酸
Lot.4	クジラ軟骨由来コンドロイチン硫酸
Lot.5	サメ軟骨由来コンドロイチン硫酸

[0035] Specified quantity weighing capacity of amino-acid-analysis each sample (Lot.1-2) was carried out, respectively, and it hydrolyzed according to about 100 degrees C and the conditions of 6 hours using 2.4 N-HCl. The quantum of this was carried out with amino-acid-analysis equipment (JLC-500V, JEOL make). The analysis result was shown in drawing 1 . Gly (glycine), HyPro (hydroxyproline), and Pro (proline) which are looked at by Lot.1 show existence of a collagen. Moreover, in Lot.2 to which purification processing was performed, since any amino acid other than GalNH2 was not seen, the aminosugar which constitutes Rajiformes cartilage origin chondroitin sulfate has ******(ed) that other amino acid and aminosugar were thoroughly removable with purification processing of that it is only a galactosamine kind and this experiment.
[0036] The 400MHz 1 H-NMR spectrum of 1 H-NMR measurement each sample (Lot.1-5) was measured. The measurement result was shown in drawing 2 . The signal of the protein origin has been checked near 0.8-2.5 ppm at Lot 1. It is thought that this protein is mainly a collagen. The spectrum of the Rajiformes cartilage origin chondroitin sulfate of Lot 2 has checked that it was close to the spectrum of the salmon cartilagines-nasi origin chondroitin sulfate of Lot 3 rather than the spectrum of Lot4 and Lot5. When this Lot 2 was looked at in the detail, the signal (GalNAC-4S) of the proton of the 4th place of the N-acetyl galactosamine considered that the sulfuric-acid radical has joined together has checked near 4.8 ppm. Moreover, although integrated intensity fell, the signal (GalNAC-6S) of the proton of the 6th place has checked it near 4.1 ppm similarly.
[0037] Disaccharide HPLC analysis 1 Chondroitin sulfate dialytic ferment (Chodroitinase ACII) 5unit was dissolved in 500micro (pH6.0) of 0.02M sodium acetate buffer solutions I.
2) The chondroitin sulfate dialytic ferment (Chodroitinase ACII) solution which dissolved in 500micro (pH6.0) of 0.02M sodium acetate buffer solutions I, and adjusted Rajiformes cartilage origin chondroitin sulfate (sample

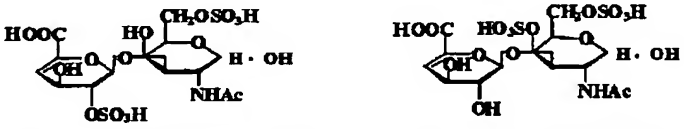
Lot.2) by 1 was added, and it stirred at 37 degrees C for 24 hours.
3) At-long-intervals alignment separation of this was carried out for 10,000rpmx 10 minutes, and supernatants were collected and it freeze-dried.
4) HPLC analysis of the obtained disaccharide oligomer was performed and the presentation of a configuration disaccharide was computed from the obtained chromatogram. The presentation ratio of a configuration partial saturation disaccharide was shown in a table 2. In addition, each structure expression is as follows. In addition, the conditions of HPLC analysis were shown in a table 3.

[0038]

[Formula 1] The structure expression of the Rajiformes cartilage origin chondroitin sulfate configuration disaccharide



Chondro ΔDi 0S Chondro ΔDi 4S Chondro ΔDi 6S



Chondro ΔDidi6S-UA2S Chondro ΔDidi4,6S

[0039]

[A table 2]

表２ 不飽和二糖構成比（%）

Condro ΔDi 0S	10.0
Condro ΔDi 4S + Condro ΔDi 6S	2.1
Condro Δdidi 6S-UA2S	80.2
Condro Δdidi 4,6S	7.7

[0040]

[A table 3]

表３ ＨＰＬＣ分析条件

カラム	GL Science NUCLEOSIL 100-5NH_2(4.6×250mm)
溶出液	２％アセトニトリル, 12-408mM NaH_2PO_4 gradient/MiliQ water
流速	0.6ｍｌ/分
試料容積	40μｌ
温度	４０℃
検出方法	ＵＶ 232ｎｍ
測定システム	HPLC(Shimadzu Corporation)

[0041] The structure of Rajiformes cartilage origin chondroitin sulfate became clear [that 2 sugar repeat unit which consists of sulfation glucuronic acid the 2nd place has the 6th place of the very characteristic presentation which occupies the 80% or more with sulfation N-acetyl galactosamine]. the above-mentioned passage -- a shark -- it is supposed that it is mainly cartilage origin chondroitin sulfate chondroitin sulfate C, and the chondroitin sulfate of this invention has the 6th place of sulfation N-acetyl galactosamine and the characteristic structure which includes specifically many 2 sugar repeat units which consist of sulfation glucuronic acid the 2nd place.

[0042] Example 3: The Rajiformes cartilage origin chondroitin sulfate or the mucopolysaccharide protein complex (following, KCS) of functional this invention of ray cartilage origin chondroitin sulfate has unique structure as

compared with the structure of the conventional known. Therefore, possibility of having the new bioactive operation which is not in other chondroitin sulfate can be considered. In order to examine this, the animal trial performed functional assessment by making into the charge of a sample offering the mucopolysaccharide protein complex containing the ray cartilage origin chondroitin sulfate prepared in the example 1.

[0043] Mixed feed administration of the KCS added to with an anticancerous of 4 weeks old BDF1 and CDF1 mouse at feed was carried out by continuation for 42 days. The tumor cell was transplanted at a rate of 1x106 cells / 0.05ml / mouse on the 28th (calcium 755 are BDF1 and Colon26 are CDF1), and administration of KCS was continued after transplantation. Cervical-vertebra dislocation of the mouse was carried out under anesthesia, a neoplasm was extracted, and wet weight was measured on the next day [KCS administration termination] (the 43rd day). The experimental plot was shown in a table 4. The multiplex comparison of Dunnett performed significant difference assay. The obtained result was shown in drawing 3 and drawing 4 . About mouse mammary gland cancer (calcium 755), significant antitumor action was statistically accepted [colon cancer / (Colon 26) / more than KCS 6% and / mouse] by administration beyond KCS 3% as compared with the control group.

[0044]

[A table 4]

表4 抗腫瘍試験の群構成

群	添加量	投与日数	動物数（匹）	
	%（混餌）	日	Ca755	Colon26
対照	0	42	8	8
KCS	1	42	8	8
〃	3	42	8	8
〃	6	42	8	8
〃	9	42	8	8

[0045] Mixed feed administration of the KCS added at feed to the ddY system male mouse of 4 weeks old of anti-MRSA (methicillin resistant Staphylococcus aureus) was carried out by continuation for 42 days. To the mouse, at a rate of 6mg / animal, it continued for 1 three days once per day, and cyclophosphamide (EX) was injected intraperitoneally from the 25th day. You made it infected by administering MRSA intravenously at a rate of 107 piece / animal at the next day which prescribed the cyclophosphamide on the 3rd for the patient. It observed until the animal died from the MRSA inoculation day, and survival time was computed. KCS continued administration within the limit of ten days until the animal died. The experimental plot was shown in a table 5. Significant difference assay performed survival analysis assay of Kaplan-Meier. The obtained result was shown in drawing 5 . In the result of survival analysis assay, the significant difference was not accepted in each test section. However, although it was 50% in the control group when seen with the survival rate of three days after, with the mouse made to take in KCS6%, 87.5%, with the mouse made to take in KCS 9%, it is 100% and some prolongation-of-life effectiveness was seen.

[0046]

[A table 5]

表5 抗MRSA試験の群構成

群	添加量	投与日数	動物数
	%（混餌）	日	匹
対照	0	死亡するまで（最大10日）	8
KCS	1	死亡するまで（最大10日）	8
〃	3	死亡するまで（最大10日）	8
〃	6	死亡するまで（最大10日）	8
〃	9	死亡するまで（最大10日）	8

[0047] The group non-taken a measure gave the guinea pig of 4 weeks old of moistness of the skin, and normal feed and a control group gave vitamin C deficiency feed. The feed which blended KCS with vitamin C deficiency feed was given to the KCS group, and it bred for 28 days, respectively. The regions-of-back skin was exfoliated after breeding for 28 days, and the collagen content was measured. Collagen content measurement ground the skin dried at 60 degrees C overnight, and hydrolyzed it 110 degrees C for 24 hours using the heating block (Yamato Science, HF21) in 6M hydrochloric acid. The hydroxyproline (Hyp) content was measured with the

chloramine-T method by making this into a sample. The experimental plot was shown in a table 6. The multiplex comparison of Dunnett performed significant difference assay. The obtained result was shown in drawing 6 . Collagens are the main constituents in the skin and are contained 70 to 80% per unit dry weight in dermis. Moreover, since reduction of a skin collagen content is also known with aging, a collagen maintains the elasticity of the skin and it is thought that it has functions, such as preventing a wrinkling. As a result of breeding a guinea pig with vitamin C deficiency feed, the collagen content in the skin decreased about 50%. On the other hand, the control inclination of collagen content lowering was accepted with the guinea pig made to take in KCS 3%.

[0048]

[A table 6]

表6 皮膚機能に関する試験の群構成

群	添加量	投与日数	動物数
	%（混餌）	日	匹
無処置	0	28	5
対照	0	28	5
KCS	1	28	5
	3	28	5

[Translation done.]

* NOTICES *

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

DESCRIPTION OF DRAWINGS

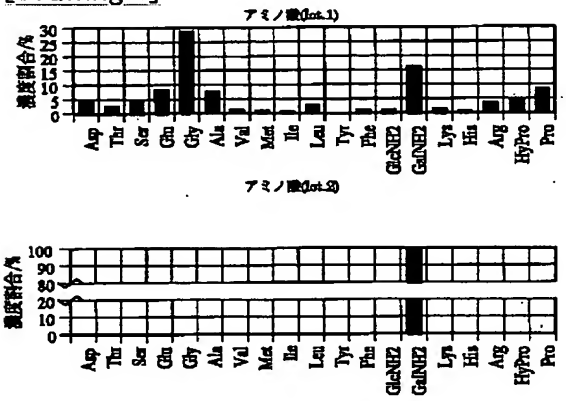
[Brief Description of the Drawings]
[Drawing 1] it is contained in each sample -- it is the graph which shows the concentration rate of amino acid variously.
[Drawing 2] It is the 400MHZ(s) 1 H-NMR spectrum chart of each sample.
[Drawing 3] It is the graph which shows the effectiveness (relation between a KCS addition and neoplasm wet weight) over the mammary gland cancer Adenocarcinoma755 of KCS.
[Drawing 4] It is the graph which shows the effectiveness (relation between a KCS addition and neoplasm wet weight) over the colon cancer Colon26 of KCS.
[Drawing 5] It is the graph which shows the effectiveness (relation of the MRSA infection days about two or more examples and the number of survival animals from which a KCS addition differs) over MRSA of KCS.
[Drawing 6] It is the graph which shows the effectiveness (relation between a KCS addition and a skin collagen content) over the skin function of KCS.

[Translation done.]

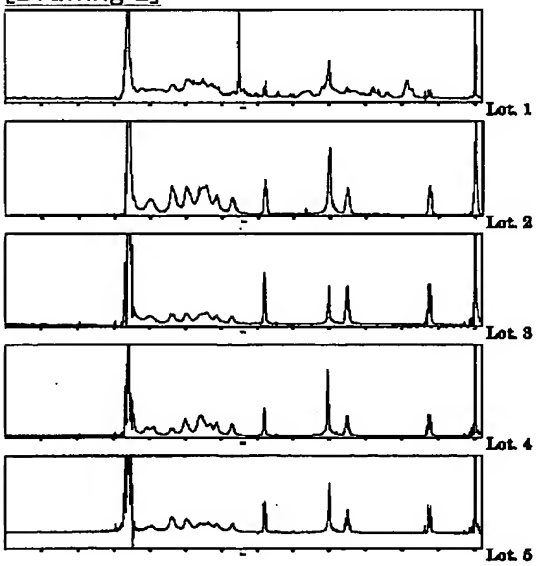
* NOTICES *

DRAWINGS

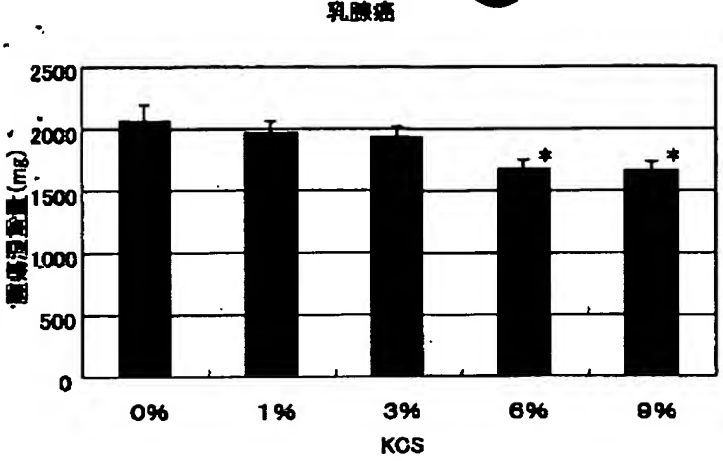
[Drawing 1]



[Drawing 2]

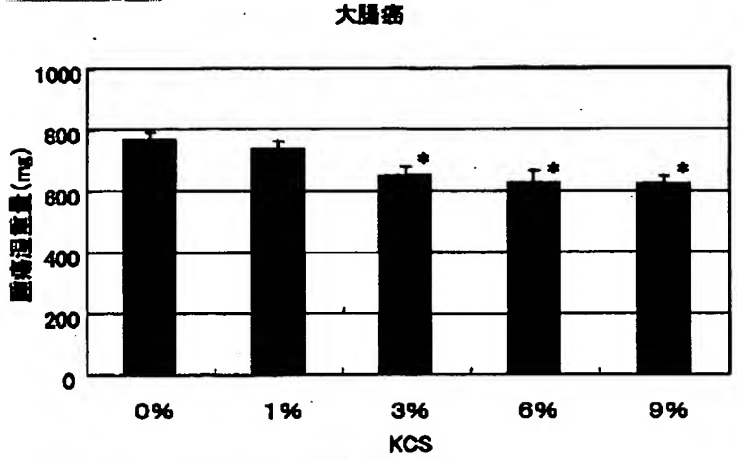


[Drawing 3]



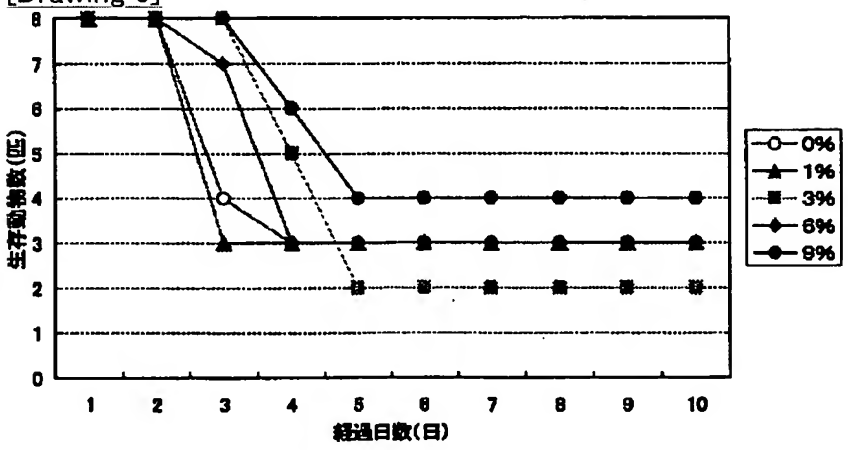
8例のBDF1マウスにおける平均値±標準偏差
*p<0.05，各被験物質投与群は，Dunnettの多重比較検定で有意差あり

[Drawing 4]

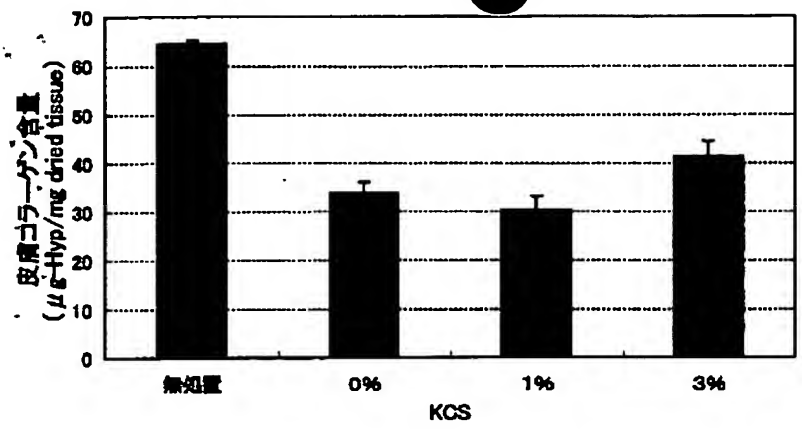


8例のCDF1マウスにおける平均値±標準偏差
*p<0.05，**p<0.01，各被験物質投与群は，Dunnettの多重比較検定で有意差あり

[Drawing 5]



[Drawing 6]



[Translation done.]

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(54)【発明の名称】　エイ軟骨由来コンドロイチン硫酸とその製造方法

(57)【要約】
【解決課題】　エイ類軟骨の高付加価値的な利用及び新規生理活性物質の提供
【解決手段】　エイ類軟骨からコンドロイチン硫酸またはムコ多糖タンパク複合体を取り出し、かかるコンドロイチン硫酸が特異な構造と生理活性作用を有することを見出した。

1

【特許請求の範囲】

【請求項１】　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸。

【請求項２】　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位（８０．２±１６．０）％、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（１０．０±３．０）％、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（２．１±０．７）％、Ｃ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（７．７±２．３）％を含む請求項１に記載のコンドロイチン硫酸。

【請求項３】　非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位、Ｃ４、Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンを含む二糖単位を含む二糖単位がランダムに連結した構造を有する請求項１または２に記載のコンドロイチン硫酸。

【請求項４】　エイ軟骨から得られる請求項１乃至３のいずれかに記載のコンドロイチン硫酸。

【請求項５】　（Ａ）Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸と（Ｂ）タンパク質とを含むムコ多糖タンパク複合体。

【請求項６】　（Ａ）Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位（８０．２±１６．０）％、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（１０．０±３．０）％、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（２．１±０．７）％、Ｃ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（７．７±２．３）％を含む請求項１に記載のコンドロイチン硫酸と（Ｂ）タンパク質とを含む請求項５に記載のムコ多糖タンパク複合体。

【請求項７】　エイ軟骨から得られる請求項５または６に記載のコンドロイチン硫酸を含むムコ多糖タンパク複合体。

【請求項８】　エイ軟骨を粉砕し、酸、アルカリまたは酵素で処理し、得られた消化液を乾燥粉末化させることを特徴とする、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸の製造方法。

【請求項９】　エイ軟骨を粉砕し、酸、アルカリまたは酵素で処理し、得られた消化液を乾燥粉末化させることを特徴とする、（Ａ）Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸と（Ｂ）タンパク質とを含むムコ多糖タンパク複合体の製造方法。

【請求項１０】　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸、または、前記コンドロイチン硫酸とタンパク質とを含むムコ多糖タンパク複合体を含む医薬品、化粧品または食品。

【請求項１１】　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸を有効成分として含み、抗腫瘍作用、免疫附活作用及び皮膚コラーゲンの減少抑制などの

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機能を有することを特徴とするコンドロイチン硫酸含有医薬品、化粧品または食品。

【発明の詳細な説明】

【０００１】

【発明の属する技術分野】本発明は、エイ類の軟骨を原料として得られる新規なコンドロイチン硫酸、このコンドロイチン硫酸とタンパク質とを含むムコ多糖タンパク複合体、これらの製造方法、及びその用途に関する。

【０００２】

【従来の技術】板鰓魚類に属するエイ類の漁獲量は、農林水産省の漁業・養殖業生産統計年報によれば全国合計で４０００ｔを超え、その約半分は北海道近海で漁獲されている。しかしながら、エイ類で食用として利用できるのは筋肉質が多い魚体のヒレ部分のみであるため、せいぜい体重の約３０％程度であり、その他の部位は軟骨が多いため食用に適さず、これまで充分有効に利用されてはいないのが現状である。

【０００３】板鰓魚類の軟骨には、生理活性物質であるコンドロイチン硫酸(ムコ多糖類)が多量に含まれており、板鰓魚類の一種であるサメ類の軟骨あるいはそれから単離したコンドロイチン硫酸は、医薬品、食品添加物、健康食品等として利用されている。また、魚類以外にも鯨、牛、羊、豚、鳥などの軟骨からも各種コンドロイチン硫酸が抽出され、利用されている。

【０００４】これらのコンドロイチン硫酸はD-グルクロン酸と硫酸化されたN-アセチルガラクトサミンの二糖繰り返し構造からなり、動物組織内ではタンパク質と結合したプロテオグリカンとして存在し、細胞外マトリクスとして多くの生理活性作用を担っている。そのため、コンドロイチン硫酸の構造も動物種や存在部位によって異なると考えられ、実際にサメ軟骨中にはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（コンドロイチン硫酸Ｃ）、鯨軟骨にはＣ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（コンドロイチン硫酸Ａ）、豚皮にはＣ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－イズロン酸からなる二糖単位（コンドロイチン硫酸Ｂ）など特異的な分布を示している。

【０００５】サメ類と同じ板鰓魚類に属するエイ類には多量の軟骨があり、この軟骨中にも生理活性物質のコンドロイチン硫酸が約５％程度含まれている。しかし、エイ類の軟骨は、非可食部の内臓等と共にせいぜい有機肥料として利用される程度であって実質的には廃棄処理されているのが実状であり、このコンドロイチン硫酸の組成構造及びそれらの機能は明らかにされておらず、産業上利用されていなかった。

【０００６】

【発明が解決しようとする課題】本発明の課題は、エイ類の軟骨から抽出した新規コンドロイチン硫酸の組成構造及びそれらの機能を明らかにし、その製造方法を示すことにより、廃棄物から低コストで大量に製造でき、様々な用途に使用できる新規コンドロイチン硫酸とその製造方法を提供することにある。

【０００７】

【課題を解決するための手段】本発明者は上記課題に鑑みて鋭意検討した結果、エイ軟骨を粉砕し、酸、アルカリまたは酵素で処理し、得られた消化液を必要に応じて中和脱塩処理した後、これをフィルタープレスなどで濾過し清澄化処理後、乾燥固化または乾燥粉末化させることによって目的とするコンドロイチン硫酸が特異な組成を有し、生理学的機能を有することを見出し、本発明を完成するに至った。

【０００８】すなわち、本発明は、エイ類軟骨から得られる新規構造及び機能を有する以下のコンドロイチン硫酸、ムコ多糖タンパク複合体、これらの製造方法及びこれらを含む食品等を提供することにある。

【０００９】（１）　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸。

【００１０】（２）　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位（８０．２±１６．０）％、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（１０．０±３．０）％、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（２．１±０．７）％、Ｃ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（７．７±２．３）％を含む前記１に記載のコンドロイチン硫酸。

【００１１】（３）　非硫酸化Ｎ一アセチル一Ｄ一ガラクトサミンを含む二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位、Ｃ４、Ｃ６位二硫酸化Ｎ一アセチル一Ｄ一ガラクトサミンを含む二糖単位を含む二糖単位がランダムに連結した構造を有する前記１または２に記載のコンドロイチン硫酸。

（４）　エイ軟骨から得られる前記１乃至３のいずれかに記載のコンドロイチン硫酸。

【００１２】（５）　（Ａ）Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫

酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸と（Ｂ）タンパク質とを含むムコ多糖タンパク複合体。

【００１３】（６）　（Ａ）Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位（８０．２±１６．０）％、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（１０．０±３．０）％、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（２．１±０．７）％、Ｃ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位（７．７±２．３）％を含む前記１に記載のコンドロイチン硫酸と（Ｂ）タンパク質とを含む前記５に記載のムコ多糖タンパク複合体。

（７）　エイ軟骨から得られる前記５または６に記載のコンドロイチン硫酸を含むムコ多糖タンパク複合体。

【００１４】（８）　エイ軟骨を粉砕し、酸、アルカリまたは酵素で処理し、得られた消化液を乾燥粉末化させることを特徴とする、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸の製造方法。

【００１５】（９）　エイ軟骨を粉砕し、酸、アルカリまたは酵素で処理し、得られた消化液を乾燥粉末化させることを特徴とする、（Ａ）Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸と（Ｂ）タンパク質とを含むムコ多糖タンパク複合体の製造方法。

【００１６】（１０）　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸、または、前記コンドロイチン硫酸とタンパク質とを含むムコ多糖タンパク複合体を含む医薬品、化粧品または食品。

【００１７】（１１）　Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含むコンドロイチン硫酸を有効成分として含み、抗腫瘍作用、免疫附活作用及び皮膚コラーゲンの減少抑制などの機能を有することを特徴とするコンドロイチン硫酸含有医薬品、化粧品または食品。

【００１８】

【発明の実施の形態】本発明のコンドロイチン硫酸は、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を６０％以上含み、さらに、非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、及びＣ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位を含む。また、本発明のムコ多糖タンパク複合体は、上記コンドロイチン硫酸とタンパク質との複合体である。

【００１９】本発明のコンドロイチン硫酸の主成分であるＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位は、サメ軟骨から精製されたものが近年明らかとなった。サメ軟骨中のコンドロイチンは、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位で主として構成されるコンドロイチン（コンドロイチン硫酸Ｃ）であり、鯨軟骨にはＣ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位、豚皮にはＣ４位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－イズロン酸からなる二糖単位を多く含むコンドロイチン（それぞれ、コンドロイチン硫酸Ａ、コンドロイチン硫酸Ｂ）がそれぞれ多く存在していることが知られているが、Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位を特異的に多く含み、かつコンドロイチン硫酸を構成している二糖の存在比が後述の割合のものは従来知られていない。

【００２０】本発明のコンドロイチン硫酸及びムコ多糖タンパク複合体は、好ましくはエイ類の軟骨から得ることができる。エイ類の種類は特に限定されないが、例え

ば、ヒラタエイ、アカエイ、イトマキエイ、ツノカスベ、ソコガンギエイ、ガンギエイ、メガネカスベ、ドブカスベ、コモンカスベ等を用いることができる。原料となるエイ類の軟骨を分離回収する方法は、例えば特願2001－124634に記載の方法等が挙げられる。

【００２１】魚体から分離された軟骨を細かく砕き、酸、アルカリまたは酵素で処理する。軟骨の粉砕は抽出溶媒が効果的に浸透する程度であればよい。例えば、ミートチョッパー等を用いて１ｃｍ³以下に粉砕するのが好ましい。酸処理は、ｐＨ１～５の範囲が好ましい。アルカリ処理は、ｐＨ１０～１４の範囲が好ましい。酵素はプロテアーゼ等の分解酵素を用いることができる。

【００２２】得られた処理物は、必要に応じて中和脱塩処理し濾過して固形分を除いた後、乾燥固化または乾燥粉末化させる。濾過は、例えば、フィルタープレス、スクリーンメッシュ、カートリッジフィルタ等を用い、清澄な抽出液とする。濾過では取り除けない抽出液中のイオンは、抽出液に電圧を印加して行う脱塩処理、イオン交換カラム等により取り除いてもよい。濾過後、乾燥固化または乾燥粉末化させることによって目的とするコンドロイチン硫酸またはムコ多糖タンパク複合体を得る。特に高純度のコンドロイチン硫酸を得るためには、酵素消化によりタンパク質を分解し、エタノールによる沈殿、イオン交換カラム処理、メンブレンフィルターによる濾過等を行なってアミノ酸や不要なアミノ糖を完全に除けばよい。

【００２３】このようにして調製したコンドロイチン硫酸は、典型的には、以下の組成を有する。

(a)Ｃ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＣ２位一硫酸化Ｄ－グルクロン酸からなる二糖単位：８０．２±１６．０%,

(b)非硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位：１０．０±３．０%,

(c)Ｃ４位またはＣ６位一硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位：２．１±０．７%,

(d)Ｃ４，Ｃ６位二硫酸化Ｎ－アセチル－Ｄ－ガラクトサミンとＤ－グルクロン酸からなる二糖単位：７．７±２．３%。

ここで%は各構成単位のモル%である。

【００２４】本発明者らは、さらにこの新規コンドロイチン硫酸またはこれを含むムコ多糖タンパク複合体は、マウス大腸癌（Colon 26）及びマウス乳腺癌（Ca 755）に対し抗腫瘍性を有し、免疫賦活による抗ＭＲＳＡ作用、ビタミンＣ欠乏モルモットによる皮膚コラーゲン低下抑制作用を有することを見出した。従って、本発明のコンドロイチン硫酸及びこれを含むムコ多糖タンパク複合体は、例えば高齢者や病弱者、体力が衰えた者などに対しての免疫力増強剤としての機能を有し、また従来のコンドロイチン硫酸と同様に、抗炎症剤、保湿剤、関節潤滑剤、眼精疲労緩和剤、皮膚の代謝改善剤などきわめて多様な用途に活用可能なものであり、さらにその特異な構造による新規機能は、医薬品、化粧品、食品、高分子工業など広い範囲に応用できる。

【００２５】

【実施例】以下、実施例、試験例を挙げて説明するが、本発明は以下の記載に限定されるものではない。

【００２６】実施例１：エイ軟骨由来コンドロイチン硫酸（ムコ多糖タンパク複合体）の製造

斜軸ニーダー（２００Ｌ容量）にエイ軟骨粉砕物１０６ｋｇとプロテアーゼ２１０ｇを入れて５９℃で２時間撹拌することにより酵素処理を行った。引き続き９０℃に加熱し１５分間撹拌して酵素を失活させ、これに濾過助剤５ｋｇを加え均質化後、フィルタープレスで加圧濾過を行った。なお、使用した加圧濾過装置は、（株）マキノ製Ｍ８－Ｓ形（濾過面積：0.27m²、濾過容積：2.7Ｌ、材質：SUS304）である。次に、得られた抽出液を９０℃に加熱し１０分間加熱殺菌後、ディスク型スプレードライヤー（（株）坂本技研製　DA220-10S）にて噴霧乾燥させコンドロイチン硫酸（ムコ多糖タンパク複合体を含む。）の白色粉末10.6ｋｇを得た。噴霧乾燥条件は、微粒化方式：φ90ｍｍ回転ピン型ディスク、12,000rpm、入口熱風温度：１８５℃、出口排風温度：９０℃、乾燥室内圧：－１０ｍｍH_2Oで行った。

【００２７】実施例２：エイ軟骨由来コンドロイチン硫酸の精製及びその構造解析

精製コンドロイチン硫酸を、以下の手順により得て構造解析に供した。

１．脱脂

1)　５０ｇのエイ軟骨乾燥粉砕品を１０００ｍｌのビーカーに入れ、アセトン５００ｍｌを加え１０分間撹拌した。

2)　５分間放置し上澄み液を捨てた。

3)　1)～2)の操作を更に３回繰り返した。

4)　残った沈殿物を減圧デシケータにて乾燥させた。

【００２８】２．アルカリ処理

1)　脱脂済みエイ軟骨粉44.5ｇを0.2Ｍ ＮａＯＨ ８００ｍｌに溶解させた。

2)　３７℃の湯浴の中で３時間撹拌した。

3)　酢酸でｐＨ７に中和した。

３．蛋白分解酸素処理

1)　0.2Ｍ Tris-HCl緩衝液（ｐＨ7.8）１００ｍｌを加えた。

2)　酢酸カルシウムを終濃度0.02Mになるように加えた。

3)　防腐のためメタノールを５０ｍｌ加えた。

4)　蛋白分解酸素（アクチナーゼE 科研製薬）を５００ｍｇ加えた。

5)　３７℃の湯浴の中で４８時間ゆっくりと撹拌した。

【００２９】４．エタノール沈殿

1) 消化液を10,000rpm×３０分間、4℃にて遠心分離した。

2) 上澄みを0.45μmメンブランフィルターを用いて吸引濾過した。

3) 上澄みに終濃度が0.05M になるように酢酸カルシウムを加えた。

4) 酢酸でｐＨ4.5 に調整した。

5) ２倍のエタノールを滴下し、４℃のもと４８時間放置した。

【００３０】５．沈殿の洗浄・乾燥

1) エタノール液を7,000rpm×３０分間、４℃にて遠心分離した。

2) 沈殿を回収し８０%エタノール３０００ｍｌを加え、４℃にて１２時間ゆっくりと撹拌した。

3) 1,000rpm×３０分間、４℃にて遠心分離した。

4) 2)～3)の操作を繰り返した。

5) 沈殿に１００%エタノール２０００ｍｌを加え、４℃にて６時間ゆっくり撹拌した。

6) 1,000rpm×３０分間、４℃にて遠心分離した。

7) 沈殿を減圧デジゲータにて乾燥させ、粗コンドロイチン硫酸を得た。

【００３１】６．ＤＯＷＥＸ５０Ｗ×２陽イオン交換カラムの調整

1) ＤＯＷＥＸ５０Ｗ×２陽イオン交換樹脂２００ｍｌを、0.5 Ｎ ＨＣl中で２時間撹拌し、ミリキュー水にて洗浄後、0.5 Ｍ ＮａＯＨ 中で２時間撹拌した。

2) 1)を3回繰り返し、交換樹脂をプロトン保持の形とし、ミリキュー水にて洗浄した。

3) ５００ｍｌビーカーにカラムを入れ、バッチ式反応槽とした。

【００３２】７．ＤＯＷＥＸ５０Ｗ×２陽イオン交換樹脂処理

1) 得られた粗コンドロイチン硫酸をごく少量のミリキュー水に溶解させた。

2) バッチ式反応槽に１)で作製した試料を添加し、1.5時間撹拌した。反応後溶液を回収し、さらに樹脂を８００ｍｌのミリキュー水にて洗浄後、洗浄液も合わせて回収した。

4) 回収液をすぐに1ＮＮａＯＨにてｐＨ７に中和した。

８．精製

1) 中和液を脱イオン水中にて３日間透析(分子量カットオフ：１０ｋＤ)した。

2) エバポレータにて適量に濃縮した。

3) 0.2μmメンブランフィルターにより濾過後、凍結乾燥し精製コンドロイチン硫酸とした。

【００３３】上記のようにして得た精製コンドロイチン硫酸を構造解析に供した。なお、比較のため、表１に示す通り、精製コンドロイチン硫酸（表１のLot.２）の他に、実施例１で調製したムコ多糖タンパク複合体（Lot.１）及び、由来の異なる３種類のコンドロイチン硫酸（Lot.３～５）を用意した。Lot.３は鮭鼻軟骨を原料とした他はLot.２と同様に調製したものである。Lot.４及び５は市販の生化学工業（株）製標品であり、Lot.４はコンドロイチン硫酸Ａ、Lot.５はコンドロイチン硫酸Ｃである。

【００３４】

【表１】

表１：構造解析に用いた試料

Lot.1	エイ軟骨由来コンドロイチン硫酸を含むムコ多糖タンパク複合体
Lot.2	エイ軟骨由来コンドロイチン硫酸
Lot.3	サケ鼻軟骨由来コンドロイチン硫酸
Lot.4	クジラ軟骨由来コンドロイチン硫酸
Lot.5	サメ軟骨由来コンドロイチン硫酸

【００３５】アミノ酸分析

各試料（Lot. 1～2）をそれぞれ所定量秤量し、2.4N－ＨＣlを用いて約１００℃、６時間の条件により加水分解を行った。これをアミノ酸分析装置（JLC-500V、日本電子製）で定量した。分析結果を図１に示した。Lot.１に観られるGly（グリシン）とHyPro（ヒドロキシプロリン）、Pro（プロリン）はコラーゲンの存在を示している。また精製処理が施されたLot.２ではＧａｌＮＨ$_2$以外のアミノ酸が観られないことから、エイ類軟骨由来コンドロイチン硫酸を構成するアミノ糖はガラクトサミン種のみであること、当実験の精製処理により他のアミノ酸、アミノ糖を完全に除去できたとが確認できた。

【００３６】^{1}H-NMR測定

各試料(Lot. １～５)の４００ＭＨｚ ^{1}H-NMRスペクトルを測定した。測定結果を図２に示した。Lot １には0.8～2.5ｐｐｍ付近にタンパク由来のシグナルが確認できた。このタンパクは主にコラーゲンであると考えられる。Lot ２のエイ類軟骨由来コンドロイチン硫酸のスペクトルは、Lot ４及びLot ５のスペクトルよりも、Lot ３のサケ鼻軟骨由来コンドロイチン硫酸のスペクトルに近いことが確認できた。このLot ２について詳細に見てみると、硫酸基が結合していると思われるＮ－アセチルガラクトサミンの、４位のプロトンのシグナル(GalNAC-4S)が4.8ｐｐｍ付近に確認できた。また面積強度は落ちるが、同様に６位のプロトンのシグナル(GalNAC-6S)が4.1ｐｐｍ付近に確認できた。

【００３７】二糖ＨＰＬＣ分析

1) コンドロイチン硫酸分解酵素(Chodroitinase ACII)

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5unitを0.02M酢酸ナトリウム緩衝液(ｐＨ6.0)500μｌに溶解した。

2)　エイ類軟骨由来コンドロイチン硫酸（試料Lot.２）を、0.02M酢酸ナトリウム緩衝液(ｐＨ6.0)500μｌに溶解し、1)で調整したコンドロイチン硫酸分解酵素(Chodroitinase ACII)溶液を加え、３７℃で２４時間撹拌した。

3)　これを10,000rpm×10分間遠心分離し、上澄を回収して凍結乾燥した。

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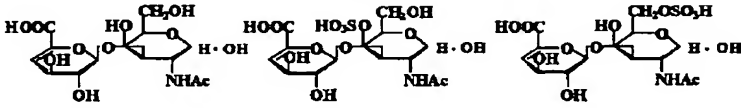
＊4)　得られた二糖オリゴマーのＨＰＬＣ分析を行い、得られたクロマトグラムから、構成二糖の組成を算出した。構成不飽和二糖の組成比を表２に示した。なお、それぞれの構造式は以下の通りである。なお、ＨＰＬＣ分析の条件は表３に示した。

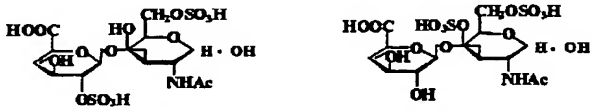
【００３８】

【化１】エイ類軟骨由来コンドロイチン硫酸構成二糖の構造式

＊



Chondro ΔDi 0S　　Chondro ΔDi 4S　　Chondro ΔDi 6S



Chondro ΔDidi6S-UA2S　　Chondro ΔDidi4,6S

【００３９】

【表２】

表２　不飽和二糖構成比（%）

Condro ΔDi 0S	10.0
Condro ΔDi 4S + Condro ΔDi 6S	2.1
Condro Δdidi 6S-UA2S	80.2
Condro Δdidi 4,6S	7.7

※【００４０】

【表３】

※

表３　ＨＰＬＣ分析条件

カラム	GL Science NUCLEOSIL 100-5NH_2(4.6×250mm)
溶出液	２％アセトニトリル, 12-408mM NaH_2PO_4 gradient/MiliQ water
流速	0.6ｍｌ/分
試料容積	40μｌ
温度	４０℃
検出方法	ＵＶ 232ｎｍ
測定システム	HPLC(Shimadzu Corporation)

【００４１】エイ類軟骨由来コンドロイチン硫酸の構造は、６位硫酸化Ｎ－アセチルガラクトサミンと２位硫酸化グルクロン酸で構成される２糖繰り返し単位が、その８０％以上を占める極めて特徴的な組成を持つことが明らかとなった。前述の通り、サメ軟骨由来コンドロイチン硫酸は主にコンドロイチン硫酸Ｃであるとされており、本発明のコンドロイチン硫酸は、６位硫酸化Ｎ－アセチルガラクトサミンと２位硫酸化グルクロン酸で構成される２糖繰り返し単位を特異的に多く含む特徴的な構造を持つ。

【００４２】実施例３：エイ軟骨由来コンドロイチン硫酸の機能性

本発明のエイ類軟骨由来コンドロイチン硫酸あるいはムコ多糖タンパク複合体（以下、ＫＣＳ）は、従来既知の構造と比較して特異な構造を有している。従って、他のコンドロイチン硫酸にはない新たな生理活性作用をもつ可能性が考えられる。これを検討するため、実施例１で調製したエイ軟骨由来コンドロイチン硫酸を含むムコ多糖タンパク複合体を供試料として、動物試験によって機能性評価を行った。

【００４３】抗腫瘍性

４週齢のＢＤＦ$_1$及びＣＤＦ$_1$マウスに飼料に添加したＫ

ＣＳを４２日間連続で混餌投与した。２８日目に、腫瘍細胞を１×１０^{6} cells/0.05ｍｌ/マウスの割合で移植し（Ca 755はＢＤＦ$_{1}$、Colon 26はＣＤＦ$_{1}$）、移植後、ＫＣＳの投与を継続した。ＫＣＳ投与終了翌日（４３日目）、エーテル麻酔下にマウスを頚椎脱臼し、腫瘍を摘出して湿重量を測定した。試験区は表４に示した。有意差検定はDunnettの多重比較により行った。得られた結＊果を図３及び図４に示した。マウス乳腺癌（Ca 755）についてはＫＣＳ ６%以上、マウス大腸癌（Colon 26）についてはKCS ３%以上の投与で対照群に比較して統計学的に有意な抗腫瘍作用が認められた。

【００４４】

【表４】

表４ 抗腫瘍試験の群構成

群	添加量	投与日数	動物数（匹）	
	%（混餌）	日	Ca755	Colon26
対照	0	４２	8	8
ＫＣＳ	1	４２	8	8
〃	3	４２	8	8
〃	6	４２	8	8
〃	9	４２	8	8

【００４５】抗ＭＲＳＡ（メチシリン耐性黄色ブドウ球菌）

４週齢のｄｄＹ系雄性マウスに飼料に添加したＫＣＳを４２日間連続で混餌投与した。２５日目よりマウスにシクロホスファミド（EX）を６ｍｇ/動物の割合で１日１回３日間連続して腹腔内投与した。３日目のシクロホスファミドを投与した翌日に、ＭＲＳＡを１０^{7}個/動物の割合で静脈内投与することにより感染させた。ＭＲＳＡ接種日より動物が死亡するまで観察を行い、生存日数を算出した。ＫＣＳは、動物が死亡するまで１０日間を限※度に投与を継続した。試験区は表５に示した。有意差検定はKaplan-Meierの生存分析検定を行った。得られた結果を図５に示した。生存分析検定の結果では、各試験区間で有意差は認められなかった。しかし、３日後の生存率でみると、対照群では50%であったが、ＫＣＳ６%を摂取させたマウスでは87.5%、ＫＣＳ ９%を摂取させたマウスでは１００%であり、若干の延命効果がみられた。

【００４６】

【表５】

表５ 抗 MRSA 試験の群構成

群	添加量	投与日数	動物数
	%（混餌）	日	匹
対照	0	死亡するまで（最大 10 日）	8
ＫＣＳ	1	死亡するまで（最大 10 日）	8
〃	3	死亡するまで（最大 10 日）	8
〃	6	死亡するまで（最大 10 日）	8
〃	9	死亡するまで（最大 10 日）	8

【００４７】皮膚の保湿性

４週齢のモルモットを、無処置群は正常飼料、対照群はビタミンC欠乏飼料を与えた。ＫＣＳ群には、ビタミンC欠乏飼料にＫＣＳを配合した飼料を与え、それぞれ２８日間飼育した。２８日間飼育後、背部皮膚を剥離し、コラーゲン含量を測定した。コラーゲン含量測定は、６０℃で一晩乾燥した皮膚を粉砕し、６M塩酸中でヒーティングブロック（ヤマト科学(株)、HF21）を用いて１１０℃、２４時間加水分解した。これを試料として、クロラミンT法によりヒドロキシプロリン（Hyp）含量を測定した。試験区は表６に示した。有意差検定はDunnettの多重比較により行った。得られた結果を図６に示した。コラーゲンは、皮膚における主要な構成成分であり、真皮中では単位乾燥重量当たり７０～８０%含有される。また、老化に伴ない皮膚コラーゲン含量の減少も知られていることから、コラーゲンは皮膚の弾力を保ち、しわを防ぐなどの機能を有すると考えられている。モルモットをビタミンC欠乏飼料で飼育した結果、皮膚中のコラーゲン含量は約50%減少した。一方、ＫＣＳ ３%を摂取させたモルモットでは、コラーゲン含量低下の抑制傾向が認められた。

【００４８】

【表６】

表６ 皮膚機能に関する試験の群構成

群	添加量	投与日数	動物数
	%（混餌）	日	匹
無処置	0	２８	5
対照	0	２８	5
ＫＣＳ	1	２８	5
	3	２８	5

【００４９】

【発明の効果】本発明のコンドロイチン硫酸及びこれを含むムコ多糖タンパク複合体は、例えば高齢者や病弱者、体力が衰えた者などに対しての免疫力増強剤として

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の機能を有し、また従来のコンドロイチン硫酸と同様に、抗炎症剤、保湿剤、関節潤滑剤、眼精疲労緩和剤、皮膚の代謝改善剤などきわめて多様な用途に活用可能なものであり、さらにその特異な構造による新規機能は、医薬品、化粧品、食品、高分子工業など広い範囲に応用できる可能性がある。

【図面の簡単な説明】

【図１】各試料に含まれるの種々アミノ酸の濃度割合を示すグラフである。

【図２】各試料の400MHz ^{1}H-NMRスペクトルチャートである。

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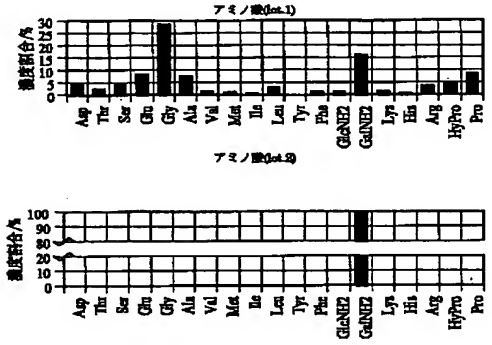
＊【図３】ＫＣＳの乳腺癌Adenocarcinoma755に対する効果（ＫＣＳ添加量と腫瘍湿重量との関係）を示すグラフである。

【図４】ＫＣＳの大腸癌Colon26に対する効果（ＫＣＳ添加量と腫瘍湿重量との関係）を示すグラフである。

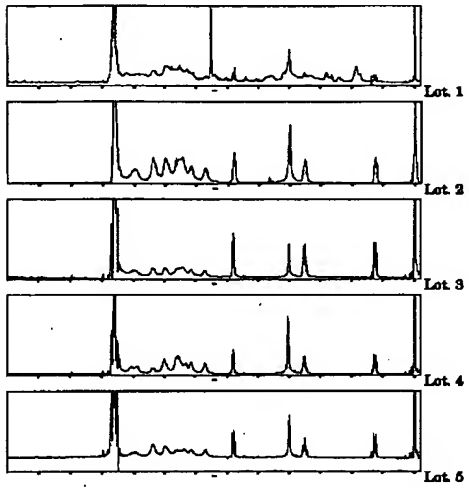
【図５】ＫＣＳのＭＲＳＡに対する効果（ＫＣＳ添加量の異なる複数の例についてのＭＲＳＡ感染日数と生存動物数との関係）を示すグラフである。

【図６】ＫＣＳの皮膚機能に対する効果（ＫＣＳ添加量と皮膚コラーゲン含量との関係）を示すグラフである。

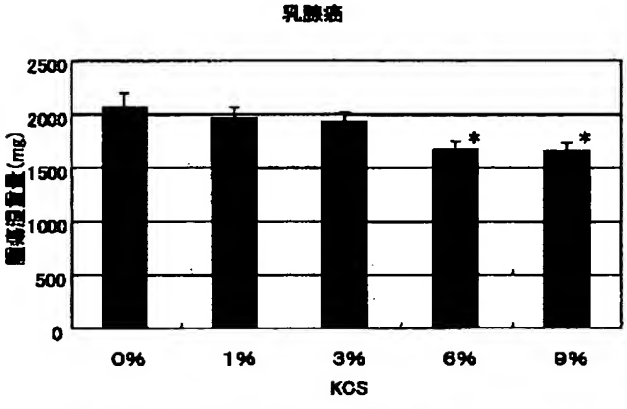
【図１】



【図２】

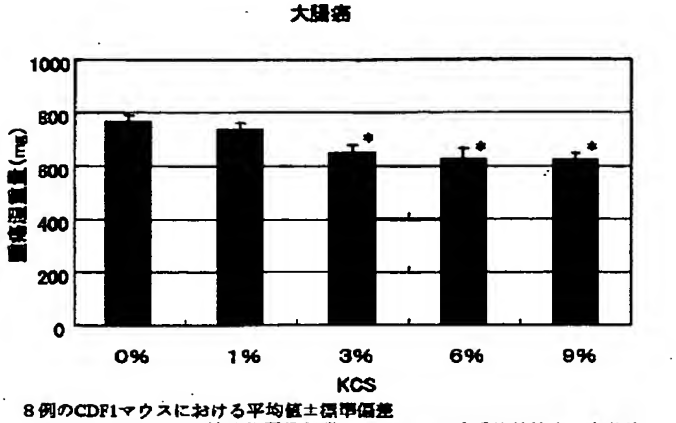


【図３】



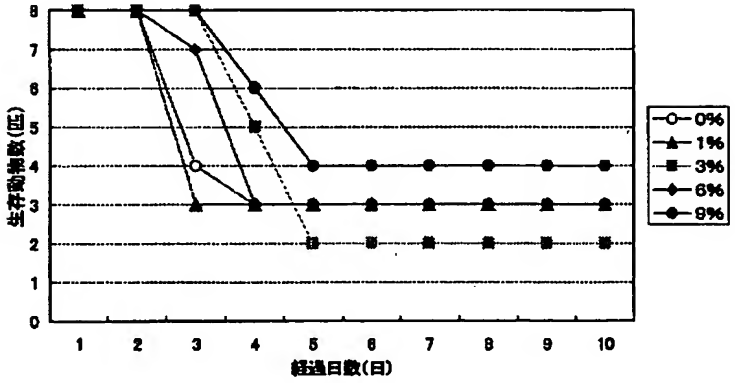
8例のBDF1マウスにおける平均値±標準偏差
*p<0.05，各被験物質投与群は，Dunnettの多重比較検定で有意差あり

【図４】

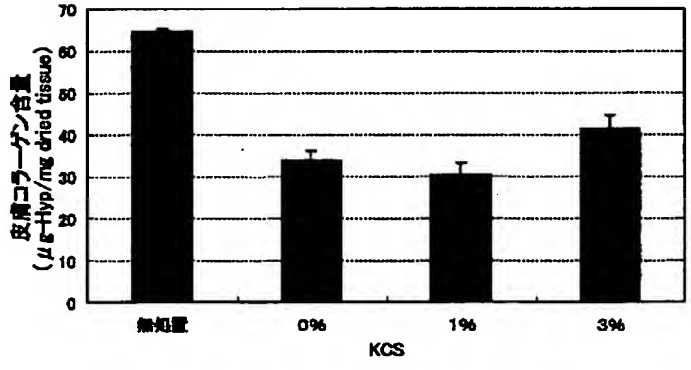


８例のCDF1マウスにおける平均値±標準偏差
*p<0.05，**p<0.01，各被験物質投与群は，Dunnettの多重比較検定で有意差あり

【図５】



【図６】



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